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CHRONIC POTASSIUM DEFICIENCY AND EXCITABILITY OF MUSCLE CELLS

Measurements with Individual Human Muscle Cells in Situ*

H.-D. Bolte and B. Lüderitz**

ABSTRACT. In four patients with chronic potassium deficiencies of various origins we found a resting membrane potential of -99.6 (s = ± 5.9) mV, a threshold current of 4.37 (s = ± 2.18) $\cdot 10^{-8}$ A and a latency time of 27.2 (s = ± 10.2) msec. In comparison to controls in healthy persons, the resting membrane potential was increased by 16.0 mV [normal: - 83.6 (s = ± 4.5) mV] ($p < 0.001$). The threshold current did not show a statistically significant change. The latency time was increased by more than 100%; i.e., by 15.3 msec [normal: 11.8 (s = ± 2.91) msec] ($p < 0.001$). Under the conditions of general potassium deficiency, therefore, an increased resting membrane potential is not necessarily linked with an increase of the threshold current or with a decrease in excitability. The increase in the latency time indicates a decrease of the ionic conductivity when the extracellular potassium concentration is diminished in general potassium deficiency. This decrease in the ionic conductivity could be an explanation for the fact that there is not necessarily a change of excitability in patients with chronic general potassium deficiency in spite of an increased resting membrane potential.

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The general adynamia which in severe cases can proceed into flaccid paralysis of the skeletal musculature is at the foreground of a distinct potassium deficiency syndrome. There is no strict correlation between the absolute serum potassium concentration and the clinical symptoms. With a rapid decrease of the serum potassium concentration, the potassium deficiency syndrome appears relatively early [7], while with a slow decrease in the serum potassium value very low concentrations (below 2.0 milliequivalents/liter) are often measured without the characteristic symptoms necessarily being demonstrable [13]. Recently, a clearly excessive resting membrane potential has been measured in individual skeletal muscle cells of patients with general chronic potassium deficiency [12]. Thus there arises the question of what role an increased resting membrane potential plays in the pathogenesis of paralyses such as are found in patients with general potassium deficiency.

The excitability of contractile biological structures is a resultant of at least 4 physiological quantities:
1. Resting membrane potential; 2. Critical membrane potential; 3. Ionic conductivity of the cell membrane; 4. Strength of excitation [10].

Under equilibrium conditions, the cell membrane has the properties of an ohmic conductor [4]. Excitation occurs with direct electrical stimulation essentially if the threshold stimulation current (I_s) depolarizes the membrane potential (E_m) to the critical potential (E_{crit}); that is, if this current provides the critical membrane depolarization ($E_m - E_{crit}$). In this process, the threshold stimulating current I_s flows through the "membrane resistance" (= "input resistance") (R). Thus we obtain the formulation

$$I_s = \frac{E_m - E_{crit}}{R}$$

Thus we can in this aspect neglect "active" sub-threshold membrane potential changes ("activation of the sodium system"). If we stimulate a skeletal muscle cell electrically and directly, i. e., with an intracellular microelectrode, with a square-wave stimulating current of long duration (more than 40 msec), then the threshold stimulating current can be determined as the lowest current that can directly produce an action potential. Depending on the size of the membrane resistance (see above) a long latent time can be measured with high membrane resistance (low ionic conductivity) and a short latent time with low membrane resistance (high ionic conductivity). The latent time is the time between the beginning of the square wave exciting current and the occurrence of the action potential.

It is possible to measure the resting membrane potential, the threshold stimulating current, and the latency time of one muscle fiber by means of a single microelectrode [3].

In the following investigations we have attempted to answer the following questions: Does general chronic potassium deficiency affect the direct muscle cell excitability as well as increasing the resting potential? 2. From measurements of the latency period of individual skeletal muscle cells under conditions of potassium deficiency, what indications for a changed ionic conductivity of the cell membrane appear?

Methods

Fundamentally, a steel canula was inserted transcutaneously into the muscle parenchyma with the aid of a special puncture apparatus. A micro-electrode was slowly advanced through this steel canula into the muscle parenchyma. The advance pressed the tip of the electrode into individual skeletal muscle cells, as could be seen from corresponding potential jumps on the screen of the cathode ray oscilloscope (for particulars, see [1,11].

Intracellular stimulation and measurement of the cellular potential were performed with one and the same glass microelectrode. Stimulation artifacts were largely avoided by use of a bridge circuit. The bridge was balanced when the microelectrode penetrated the cell membrane. Finally, starting at zero current, the strength of the stimulation was slowly increased from stimulus to stimulus (frequency: 1/second) until an action potential appeared on the screen of the oscilloscope. Square-wave currents with a duration of 40 - 50 msec were applied. The current which just surpassed the threshold was considered the threshold stimulation current. The time from beginning of stimulation to the beginning of the fast rise in action potential was the latency time. Only those cells were evaluated for which the resting potential showed stepwise changes, and for which there was no change during the bridge balancing (for particulars, see [3]).

Plasma electrolyte concentrations were determined by flame photometry. For each of the patients studied, a muscle excision (50 - 150 mg) was performed from the musculature of the Musculus tibialis anterior. The muscle samples were obtained through a skin incision about 5 cm long, under subcutaneous anasthesia with 2% Novocain. Extracts were prepared by one hour of boiling in distilled water for determination of the potassium content of the muscle sections. The potassium content of these extracts was determined by flame photometry. The tissue water content was determined from the difference between dry weight and wet weight. The measurements obtained for tissue potassium were referred to one kilogram of wet weight. The bicarbonate concentration in the plasma was determined by the method of van Slyke et al. [14].

Results

A total of 4 patients were studied. They had distinct chronic general potassium deficiency of different etiology: (Case 1: chronic chronic vomiting from stenosing Ulcus duodeni;

Case 2: chronic diarrhea from Colitis ulcerosa; Case 3: potassium-losing nephropathy; Case 4: chronic laxative abuse and chronic pyelonephritis with limitation of endogenous creatinine clearance to 67 ml/min).

The means of the potassium, sodium, bicarbonate, and calcium plasma concentrations are shown in the table.

TABLE. RESTING MEMBRANE POTENTIAL, THRESHOLD CURRENT, LATENCY TIME, AND PLASMA CONCENTRATIONS OF POTASSIUM, SODIUM, BICARBONATE AND CALCIUM IN PATIENTS WITH CHRONIC GENERAL POTASSIUM DEFICIENCY, IN COMPARISON WITH CONTROL MEASUREMENTS. MEANS WITH STANDARD DEVIATION (s) AND NUMBER OF INDIVIDUAL MEASUREMENTS (n).

	E_m (mV)	J_s ($\times 10^{-8}$A)	t_l (msec)	K (meq/l)	Na (meq/l)	HCO_3 (meq/l)	Ca (meq/l)
Normal persons	−83,6 $s = \pm$ 4,5 $n =$ 15	5,30 $s = \pm$ 0,88 $n =$ 15	11,8 $s = \pm$ 2,9 $n =$ 15	4,2 $s = \pm$0,8 $n =$ 6	141 $s = \pm$2,6 $n =$ 6	23,8 $s = \pm$2,8 $n =$ 6	4,8 $s = \pm$0,19 $n =$ 5
P	< 0,001	< 0,2	< 0,001	< 0,001	< 0,005	< 0,05	
Chronic potassium deficiency	−99.6 $s = \pm$ 5,9 $n =$ 20	4,37 $s = \pm$ 2,18 $n =$ 20	27,20 $s = \pm$10,2 $n =$ 20	2,0 $s = \pm$0,36 $n =$ 4	131 $s = \pm$4,3 $n =$ 4	29,0 $s = \pm$4,3 $n =$ 4	4,8 $s = \pm$0,23 $n =$ 3

In a total of 20 individual skeletal muscle cells of the 4 patients mentioned with chronic general potassium deficiency, we found a threshold current of 4.37 ($s = \pm 2.18$) $\cdot 10^{-8}$ A and a latency time of 27.2 ($s = \pm 10.2$) msec. In comparison to control investigations with healthy persons [3], the resting potential of -99.6 ($s = \pm 5.9$) mV was clearly increased by about 16.0 mV [normal: -83.6 ($s = \pm 4.45$) mV ($p < 0.001$) The threshold stimulating current did not show a statistically significant change. The latency time was more than doubled, by 15.3 msec [normal: 11.8 ($s = \pm 2.91$) msec ($p < 0.001$) (see also the Table). Figure 1 shows a characteristic example of our measurements, drawn from original photographs (see

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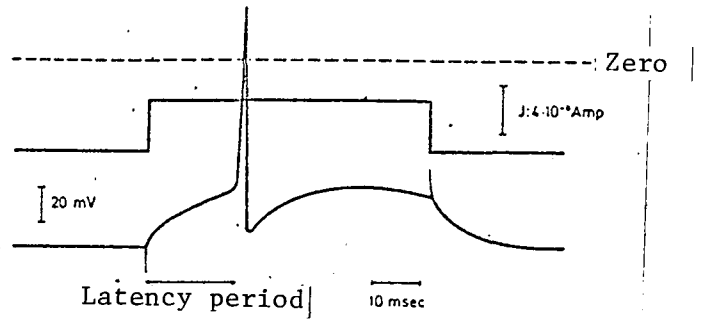

Figure 1

Measurements of resting membrane potential, latency time, and threshold current of a skeletal human muscle fiber in situ by use of a bridge circuit. This is a drawing from an original photograph made from a patient with general chronic potassium deficiency. The cell was stimulated and the membrane potential was measured with the same micro-electrode. The strength and duration of the square-wave current are shown just below the zero line, with the membrane and action potential at the bottom.

the Table also).

The general potassium deficiency in the patients studied corresponded to a decrease of the extracellular potassium concentration to 2.0 (s = $\pm$ 0.36) milliequivalents/liter of plasma. Furthermore, the potassium content of muscle tissue was determined from muscle excisions and compared with corresponding analyses from three clinically healthy specimens. In the patients with general chronic potassium deficiency, we found a tissue calcium concentration of 65.3 (s = $\pm$ 8.6) milli-equivalents/kg wet weight (total of 8 muscle samples from 4 patients) in comparison to a control result of 83.4 (s = $\pm$ 8.8) milliequivalents/kg wet weight (total of 9 muscle samples from 3 healthy persons) ($p < 0.001$). In the potassium-deficient

patients, the water content of the muscle samples was slightly greater, 78.5 (s = ± 2.6) percent of the wet weight, than in the control persons, with 75.4 (s = ± 0.9) percent of the wet weight. These findings lead to the conclusion that the chronic potassium deficiency has been expressed in the four patients studied as a lowering of the cellular potassium concentration by some 20%.

Discussion of the Results

Our test results with individual skeletal muscle cells of patients with chronic general potassium deficiency thus show a normal threshold stimulating current with clearly elevated resting membrane potential. At the same time we can demonstrate a greatly increased latency period.

The latency period of a muscle fiber is essentially the resultant of the capacitive and ohmic properties of the limiting surface of the cell. An increase in these two quantities is expressed in an increase of the latency period [9]. From numerous experimental studies it is well established that the capacitive properties of a cellular limiting surface do not change under biological conditions. This is due to the particular structural nature of the cell membrane [5]. That indicates that changes of the latency period must be related primarily to changes in the ionic conductivity of the membrane.

From investigations of the specific membrane resistance on the musculature of cold-blooded animals [6] and warm-blooded animals (rat) [2], it can be seen that a lowering of the extracellular potassium concentration is associated with an increase of the membrane resistance and so with a decrease in the total ionic conductivity. Correspondingly, the latency period is considerably increased (rat diaphragm, in vitro) [8]. A lowered membrane conductivity [= increased membrane resistance (R)] can, with other conditions being the same,

have the effect of increasing the excitability, thus leading to a decrease of the threshold current. Our finding of an unchanged threshold current in patients with chronic potassium deficiency makes it very probable that an increase of the membrane resistance has a compensatory effect on a simultaneous increase of the critical membrane depolarization ($E_m - E_{crit}$) (see Introduction). This finding could be an explanation for the clinical experience that only a small percentage of patients with chronic general potassium deficiency have paralysis.

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